The Occurrence of Thelypterin in Ferns

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Two inhibitory substances, thelypterin A and B, have been isolated from the fern *Thelypteris normalis* (C. Chr.) Moxley. High concentrations of the thelypterins inhibit cell division in *T. normalis* gametophytes, whereas at lower concentrations the gametophytes are ameristematic (Davidonis & Ruddat, 1974). Sporophyte roots and pre-elevation stage leaves (those just prior to petiole elongation and leaf uncurling) contain thelypterins A and B (Davidonis & Ruddat, 1974); the structure of neither compound is known. This paper reports the occurrence of thelypterins in the culture medium of *T. normalis* gametophytes. Also, other fern genera were examined for thelypterins.

MATERIALS AND METHODS

Spores of *T. normalis* were collected from sporophytes grown in the Wheaton College greenhouses. Spores were surface sterilized with 0.5% sodium hypochlorite and sown in flasks containing modified Knudson's medium (Steeves et al., 1955) supplemented with trace elements (Nitsch, 1951) and placed on a rotary shaker under continuous illumination at 25°C.

Expanding fern leaves were completely immersed for 90 hours in a liter of distilled water under continuous illumination at 25°C. Fern roots were prepared as previously described (Davidonis & Ruddat, 1973). Culture medium in which gametophytes had grown (conditioned culture medium) and distilled water containing leaf or root diffusion products (diffusates) were concentrated under reduced pressure in a rotary evaporator, acidified to pH 4.0, and extracted with ethyl acetate. The ethyl acetate fraction was dried, evaporated, and chromatographed on thin-layers of Silica-gel pF 254 (Merck) in ethyl acetate-isopropanol (7:3, V/V). The chromatogram was divided into six zones which were removed and eluted with methanol. One, or occasionally two, bands on the chromatogram fell into each zone. The solvent was evaporated and the fraction dissolved in distilled water and assayed by the *T. normalis* spore bioassay (Davidonis & Ruddat, 1973). The criteria for thelypterins were: a positive Ehrlich reaction for thelypterin A (pink) and no reaction for thelypterin B, co-chromatography with thelypterins, and growth inhibition in the *T. normalis* spore bioassay.

RESULTS AND DISCUSSION

One liter of culture medium in which *T. normalis* gametophytes had grown for 35 days contained thelypterin A in an amount equivalent to that found in 2.5 g of fern root dry weight (Davidonis & Ruddat, 1973). Thelypterin B was not detected. A gametophyte producing large quantities of thelypterin A can inhibit the growth of other gametophytes, thereby reducing competition.

Thelypteris normalis leaf diffusates also contain thelypterin A but not thelypterin B. Excised croziers placed on agar release both thelypterin A and B into the medium (Davidonis & Ruddat, 1974). Therefore, foliar leaching may be an important means of releasing thelypterin A into the environment.

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Nine fern species belonging to seven genera were tested for thelypterins (*Table 1*). Root diffusates were used, except for *T. noveboracensis* (L.) Nieuwl. and *Phlebodium aureum* (L.) J. Smith, where leaf diffusates were tested because these two species had small root systems. Thelypterin A was released from *T. dentata* (Forsk.) E. St. John and *T. noveboracensis*, thelypterin B from the roots of *T. dentata*, *Pteris vittata* L., and *P. multifida* Poir. *Osmunda cinnamomea* L. leaves (but not roots) contained an inhibitor different from the thelypterins. The inhibitor co-chromatographed with thelypterin A but did not give a positive Ehrlich reaction. *Cyrtomium falcatum* (L.) Presl, *Dryopteris spinulosa* (O. F. Muell.) Watt, and *Pellaea viridis* (Forsk.) Prantl contained neither the thelypterins nor other inhibitors.

TABLE 1. THELYPTERINS IN FERNS.

Species	*Source of Material*	*Thelypterin* A	B	*Other Inhibitors*
	OSMUNDACEAE			
Osmunda cinnamomea	roots	–	–	–
O. cinnamomea	leaves	–	–	+
	PTERIDACEAE			
Pellaea viridis	roots	–	–	–
Pteris multifida	roots	–	+	–
P. vittata	roots	–	+	–
	THELYPTERIDACEAE			
Thelypteris normalis	roots	+	+	–
T. dentata	roots	+	+	–
T. noveboracensis	leaves	+	–	–
	DRYOPTERIDACEAE			
Cyrtomium falcatum	roots	–	–	–
Dryopteris spinulosa	roots	–	–	–
	POLYPODIACEAE			
Phlebodium aureum	leaves	–	–	–

The presence of inhibitors that can be leached from leaves or released from roots may prevent the establishment of gametophytes in the immediate vicinity of the sporophyte. This phenomenon may account for the general absence of gametophytes near sporophytes under natural conditions.

The results in *Table 1* suggest that thelypterin A could act as a chemotaxonomic marker of phylogenetic significance within the Thelypteridaceae. A recent classification (Nayar, 1970) suggests that the Thelypteridaceae and the Pteridaceae are not closely related.

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